Attenuated Total Reflectance of Infrared Energy by Dairy Products

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Abstract

When infrared energy was reflected from the internal face of a prism in contact with some dairy products, or model systems, an attenuation was observed which related to the infrared absorption properties of the sample in contact with the reflecting face. Even though the amount of attenuation of infrared radiation of characteristic wavelength was found directly related to the concentration of butteroil dissolved in benzene, no similar effect was observed on studying infrared radiation attenuated by a variety of cheeses and by milks containing graded levels of fat. However, a significant linear relationship was found between the attenuation of infrared radiation having a wave number of 1,035 cm^{-1} and the total solids content of concentrated milk.

The procurement of data pertaining to the infrared absorption by dairy products presents special problems probably first recognized by Goulden (4). These problems rise from the intense absorption of infrared energy by the relatively large amount of water found in most dairy products and the scattering of energy by the dispersed phase of the colloidal systems.

In 1961, Fahrenfort (2) suggested that the infrared absorption characteristics of materials, unmanageable by conventional techniques, could be determined by observing the manner in which they attenuated the infrared energy reflected internally by a prism. The obvious usefulness of studying attenuated total reflectance (ATR) quickly led to the commercial development of equipment capable of converting existing infrared spectrophotometers into devices suitable for studying infrared attenuation.

Since the development of this equipment, a number of papers have been printed describing its application to the analysis of model compounds in aqueous systems and other materials (5, 7, 8). No formal study of the use of ATR for the analysis of food products has yet been published.

This paper presents a brief description of the technique as we applied it to the analysis of some dairy products, and the results obtained.

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Methods and Materials [1]

The technique of attenuated total reflectance is based on the fact that when a beam of radiant energy is totally reflected internally from a surface, as in a prism, there is an exchange of energy between the two media forming the reflecting surface. The energy exchange is roughly proportional to the ratio of the refractive indices of the materials forming the interface. In a glass prism in contact with air, the internally reflected beam suffers no attenuation, since air has a low index of refraction. However, if the material in contact with the prism surface has a refractive index the same or greater than the prism, all radiant energy will move out of the crystal and no internal reflection will occur.

The index of refraction of a material, as a function of wavelength becomes very high at those wavelengths where absorption occurs. A plot of the intensity of reflectance off a surface against the wavelength of the reflected light will, therefore, produce a spectrum very similar to the conventional transmission spectrum, since absorption by materials forming the surface will cause a drop in intensity of the reflected beam.

In practice, the sample to be analyzed is brought into optical contact with the face of a prism made of a transparent material of high refractive index. Since the attenuation at any one interface is small, better patterns can be obtained by use of prisms allowing multiple internal reflections. Our work was conducted with prisms of such design. As shown in Figure 1, a continuous spectrum of infrared radiation was internally reflected from three sample crystal interfaces. The intensity of the reflected radiation was then analyzed and recorded. A conventional infrared spectrophotometer was used to furnish the radiation beam to analyze and record the intensity of the reflected radiation. Since these data are recorded

[1] Reference to certain products or companies does not imply an endorsement by the Department over others not mentioned.

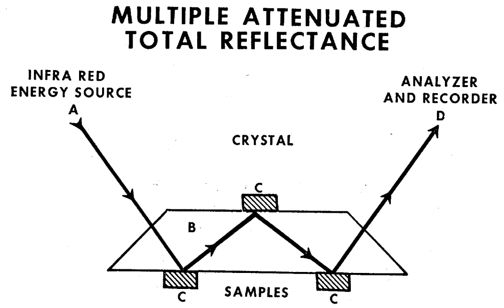

FIG. 1. Schematic diagram of energy path in ATR equipment used.

on standard spectrophotometer paper, the degree of attenuation observed can be considered in terms of transmission and units derived therefrom. Quotation marks indicate where conventional energy absorption terminology is used to indicate attenuation of reflectance.

The attenuation of the reflected beam depends only upon the index of the prism in the absorbing regions and the angle of incidence of the radiation. Therefore, in theory, the spectrum obtained is independent of sample thickness. Reproducible quantitative data related to the infrared absorption characteristics of materials should then be simply obtained by use of this technique.

An ATR unit purchased from Barnes Engineering Corporation (Commerce Road, Stamford, Connecticut) and equipped with their KRS-5 (thallous bromide-iodide) crystal was attached either to a Model 21 or 421 Perkin-Elmer infrared spectrophotometer for our studies and used as directed by the manufacturer. The angle of incidence of the energy beam was held constant at 45 degrees.

The butteroil used was obtained by melting freshly churned, sweet cream butter. Cheeses studied were both experimental samples produced in our laboratory (6) and commercial material purchased in local supermarkets. The milk concentrates were made using a falling-film evaporator, custom-built by the Arthur Harris Company (Chicago, Illinois). Since the concentrates were originally made for the manufacturer of sterilized or dehydrated samples, their initial total solids (TS) contents were approximately 25, 36, and 50%. To obtain a greater range of concentrations, some of these samples were quantitatively diluted prior to ATR analysis. The TS of all samples, prior to dilution, was determined by the standard Mojonnier technique.

Results

All spectra obtained by use of the ATR technique closely resembled those obtained by conventional infrared spectrophotometry where comparison was possible. In Fig. 2, sections of ATR spectra of butteroil in benzene are shown. The principal regions of attentuation correspond closely to the absorption bands of the triglycerides reported by Chouteau (1). The extent of attenuation depends upon the concentration

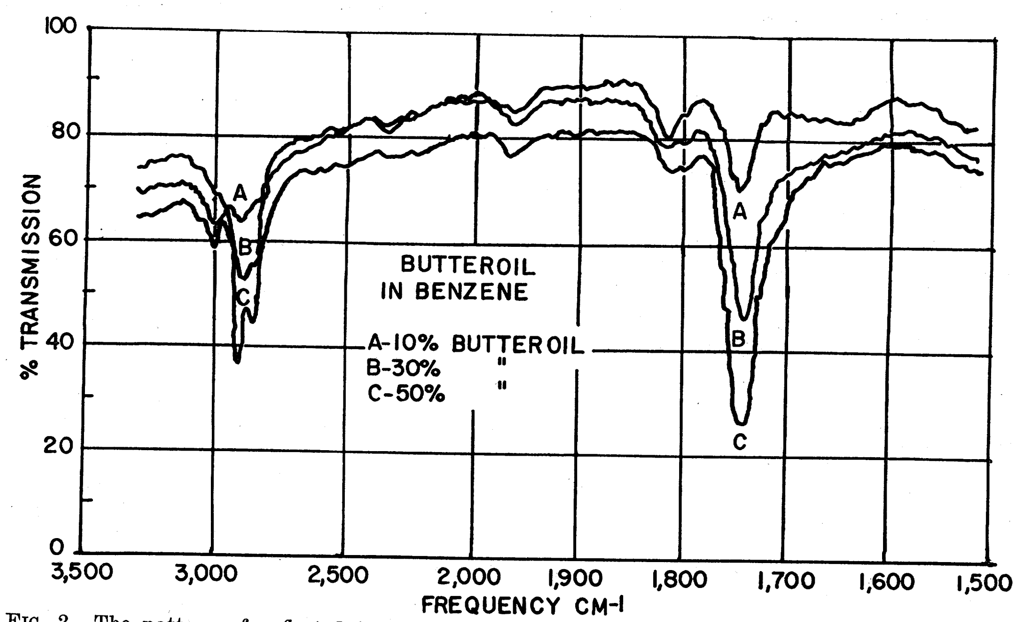

FIG. 2. The pattern of reflected infrared radiation attenuation by butteroil in benzene. A decrease in transmission is equivalent to an increase in attenuation. Perkin-Elmer Model 21 spectrometer used.

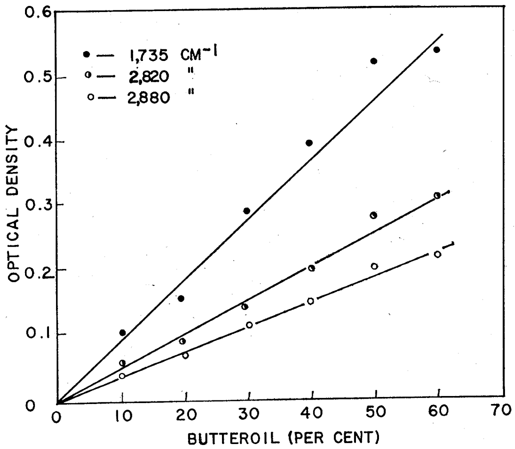

FIG. 3. Relationship between concentration of butteroil in benzene and attenuation of reflectance as expressed in optical density units derived from transmission data taken from Fig. 2.

of butteroil in the solvent and the relationship between optical density of the solution at 1,735, 2,820, and 2,880 cm^{-1} and the concentration of butteroil was found to be linear, as shown in Fig. 3.

Although the ATR spectra of fat containing milk showed characteristic attenuation at 1,735 cm^{-1}, the extent of attenuation did not directly relate to the fat content of the milk as shown in Fig. 4.

When examining the ATR spectra obtained from an analysis of a number of cheese varieties including Cheddar, Bel Paese, Liederkranz, Blue, Camembert, and experimental low-fat cheeses produced in our laboratory, the characteristic attenuation at 1,735 cm^{-1} was always observed. However, the degree of attenuation did not relate to the fat content of the cheese. The patterns of infrared attenuation by the cheeses studied were all similar to that shown in Figure 5. However, some difference in relative degree of attenuation at the various wavelengths was noted which may relate to cheese type or physical condition at time of analysis. This point was not studied in detail.

It was only upon examining the ATR spectra of milk concentrates that the observed attenuation was found to have a quantitative relationship to milk composition. The broad general attenuation at 1,035 cm^{-1} shown in Figure 6 was found to be directly influenced by the total solids in the milk. Regression analysis (3) of the data in Figure 7 showed a linear relationship between optical density of milk at 1,035 cm^{-1} and its total solids content. For the whole milk data shown, the correlation coefficient was 0.932 and the best straight line through the points had a slope of 0.0062. Similar data for skimmilk also gave a linear relationship with a correlation coefficient of 0.969 and described by a line having a slope of 0.0085 and an intercept of .120.

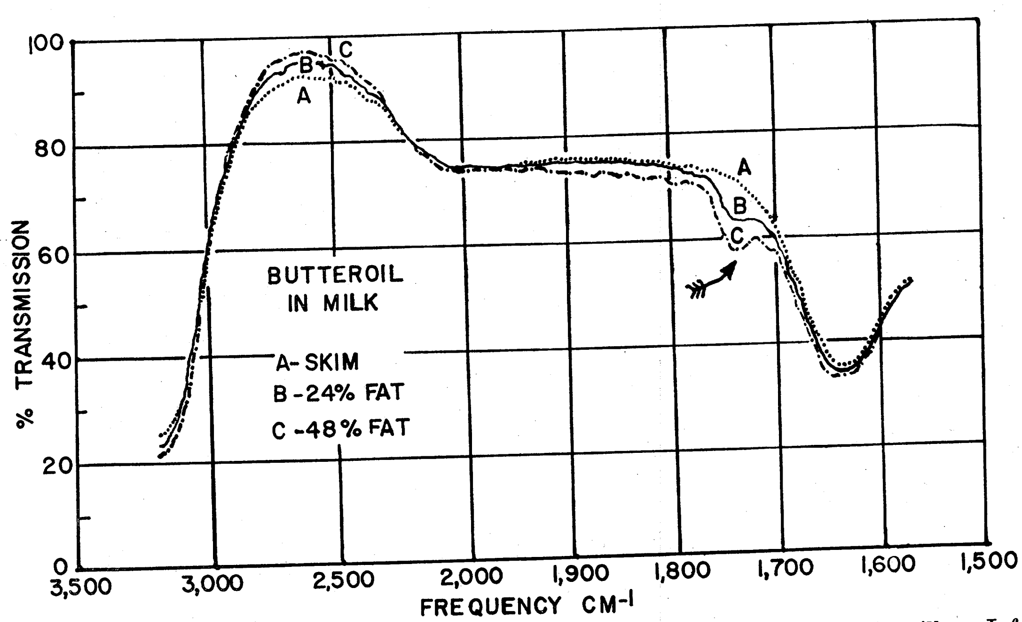

FIG. 4. Sections of ATR spectra showing the effect of increased levels of butteroil in milk on Infrared energy. Spectra obtained using Perkin-Elmer Model 21 spectrometer. Butteroil homogenized into skimmilk using 2,000 psi pressure.

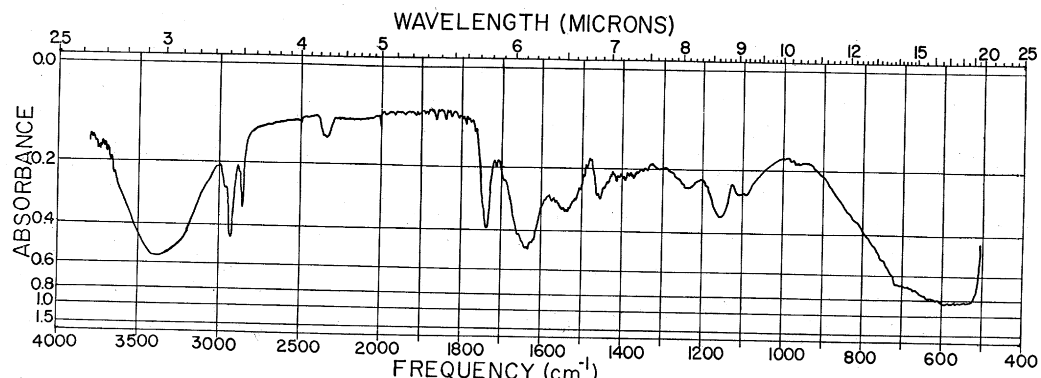

Fig. 5. Complete ATR spectrum of a commercial sample of Cheddar cheese. Data obtained using Perkin-Elmer Model 421 spectrometer.

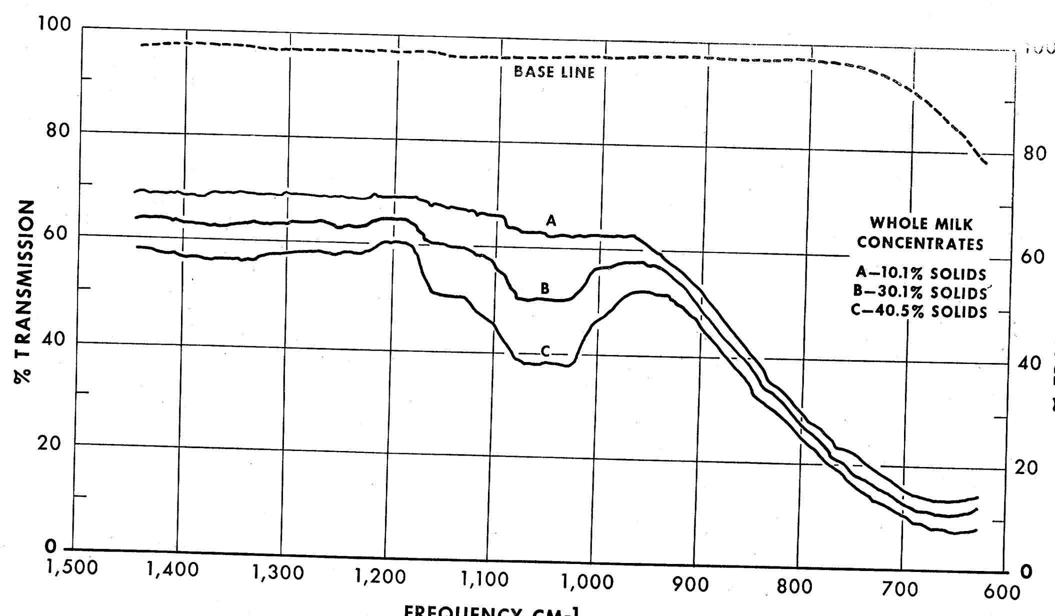

Fig. 6. Sections of ATR spectra in region where observed attenuation as expressed in terms of transmission relates to TS of whole milk concentrates. Spectra obtained using Perkin-Elmer Model 21 spectrometer.

Discussion

The observed attenuation of internally reflected infrared energy from a crystal face in contact with dairy products can be used to study their infrared absorption characteristics. However, the theoretically indicated linear relationships between the observed attenuation and the concentration of specific energy absorbing chemical groups in the systems under investigation were not observed, except in simple solutions. The observed deviations from linearity in this relationship may be ascribed to the scattering of energy by the physical structure of the multiphase dairy products.

We found that the extent of attenuation in a broad region around 1,035 cm^{-1} correlated well with the total solids in whole and skimmilk concentrates. Due to the broadness of the attenuation band in this region, it is impossible to ascribe its appearance to infrared absorption by any one milk component. All phases probably contributed to the attenuation, either by absorbing or scattering energy, since the slope of the curves relating attenuation to TS in whole milk concentrates differs from that obtained with skimmilk concentrates.

These linear relationships suggest that the observation of ATR could possibly be used to rapidly monitor the TS in the output of high speed evaporators. For this purpose, the amount

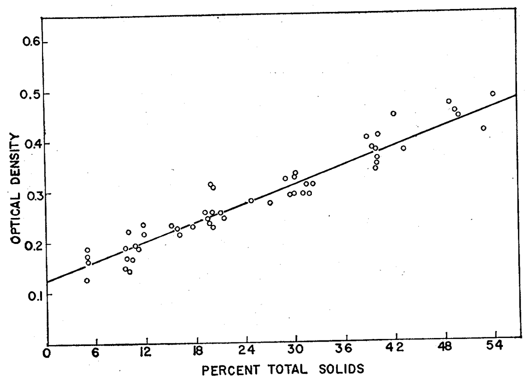

FIG. 7. Relationship between reflected infrared attenuation as expressed in terms of optical density and TS of whole milk concentrates. Line obtained by simple regression analysis.

of data scattering shown in Fig. 7 would be undesirable. However, it is probable that the scattering we observed resulted because the ATR unit used in our study was attached to the spectrometer with standard sample holder clips. This arrangement allows small uncontrollable shifts in the position of the light path, resulting in small changes in the observed attenuation. Higher levels of precision would certainly result from a more rigidly fixed light path. Likewise, more exact results could be obtained using crystals other than thallous bromide-iodide, since these tend to become cloudy when kept in contact with samples containing water. Since crystals of more water resistant materials were not available when our study was carried out, it was necessary to occasionally polish off the clouded surfaces of the crystals used. This resulted in slight changes in the geometry of the system which contributed to the observed scatter.

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